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BIOMAGNETISM AND FERRITIN

SEMI-ANNUAL REPORT

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I. INTRODUCTION

This current progress report shows some tantalizing results. There appears to be a pronounced decrease in embryonic development due to the gradient magnetic field (30.2 vs. 55.4% hatching), but the overall results have enough variability to provide only a low level of significance.

Improvements in technique had to made during the runs. This will enable us to repeat a series next spring with greater consistency and efficiency, with more runs per month to give better statistics.

The embryos from these experiments are currently being examined histologically and chemically, in an attempt to determine the nature of the arrested development.

II. PROCEDURE

Work from the beginning of the year through the end of May has consisted mainly of growing as many fertilized eggs as possible in the magnetic field with corresponding controls. One two hour old embryo was placed in each of the 13 compartments of both the sample and control holders (see Figure 1). The embryos were allowed to develop to the hatching stage in these compartments (3-4 days) while nutrient medium was circulating through them (see Figure 2). At this rate, it was possible to run up to two sets of embryos per week. Twenty embryos were placed in glass finger bowls in addition to those in each container. All embryos were examined daily in order to determine at what stage the embryos that ceased development before hatching did so. Records were kept to determine the percentages of samples, controls, and embryos in finger bowls developing to the hatching stage. Non-developing eggs were fixed in 5% formalin for subsequent biochemical study and in Bouin's Fluid for histological study. These runs were stopped at the end of May because of the seasonal loss of ability of Rana pipiens to be induced to ovulate and produce fertilized eggs.

Certain physical problems were encountered. The first were temperature control problems due to large temperature fluctuations in the room in which the magnet was first located. The second was a lack of space between the top of the holders and the upper pole of the magnet which caused the nutrient to touch the magnet and overflow occasionally (see Figure 3). On March 31, the magnet was relocated in a "constant temperature" room and the former problem was alleviated. The nutrient temperatures in the sample and control holders remained very close (0.1°C.) and stayed quite constant as a function of time from this date on (see Table 1). On April 3, the rate of the circulating pump was increased in order to insure adequate nutrient flow for the removal of wastes and contaminants from the embryos. It was thought that this would bring the rate of development of the controls up to that of the embryos in the finger bowl. From then until April 16th, the water in the egg

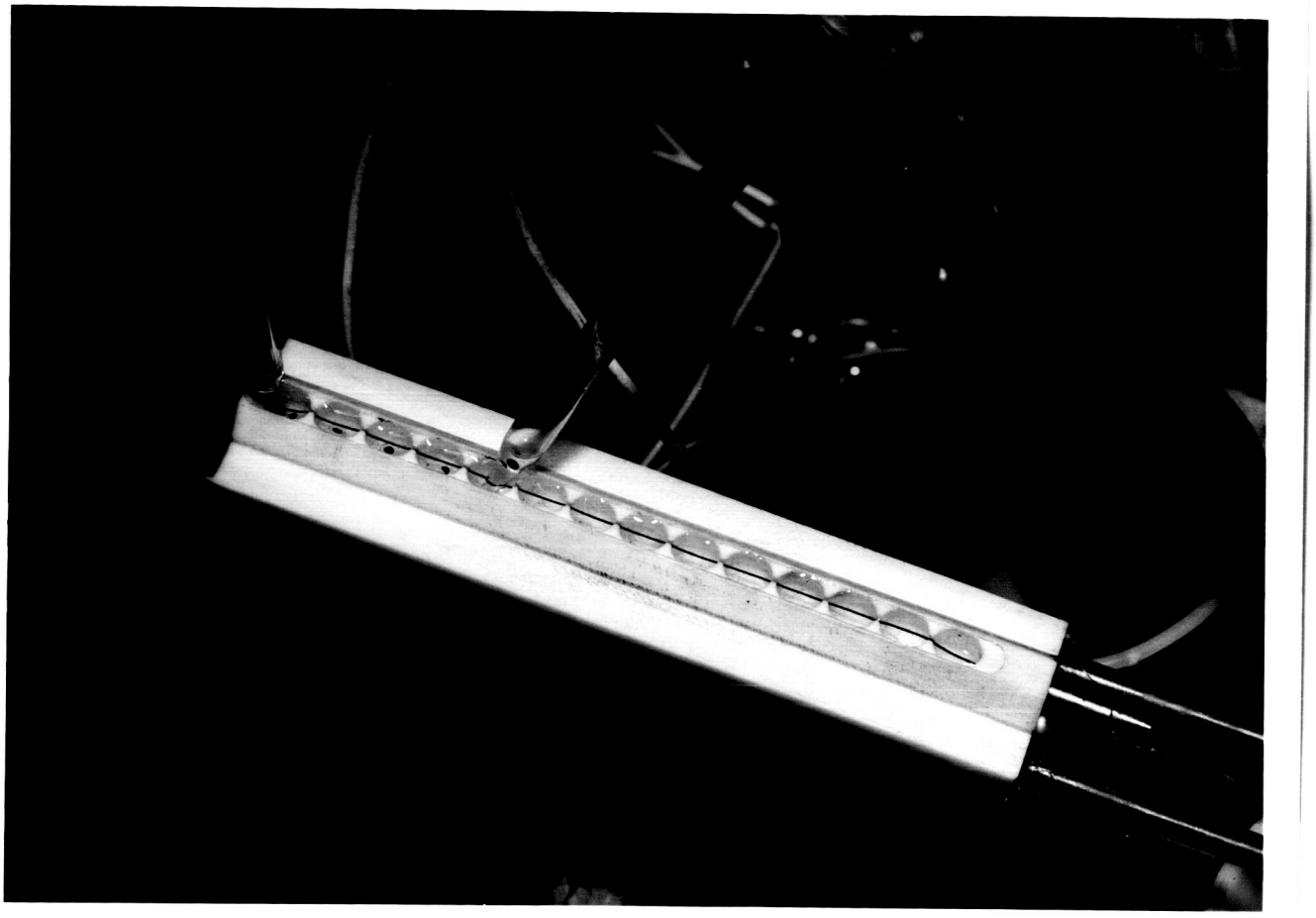

Figure 1

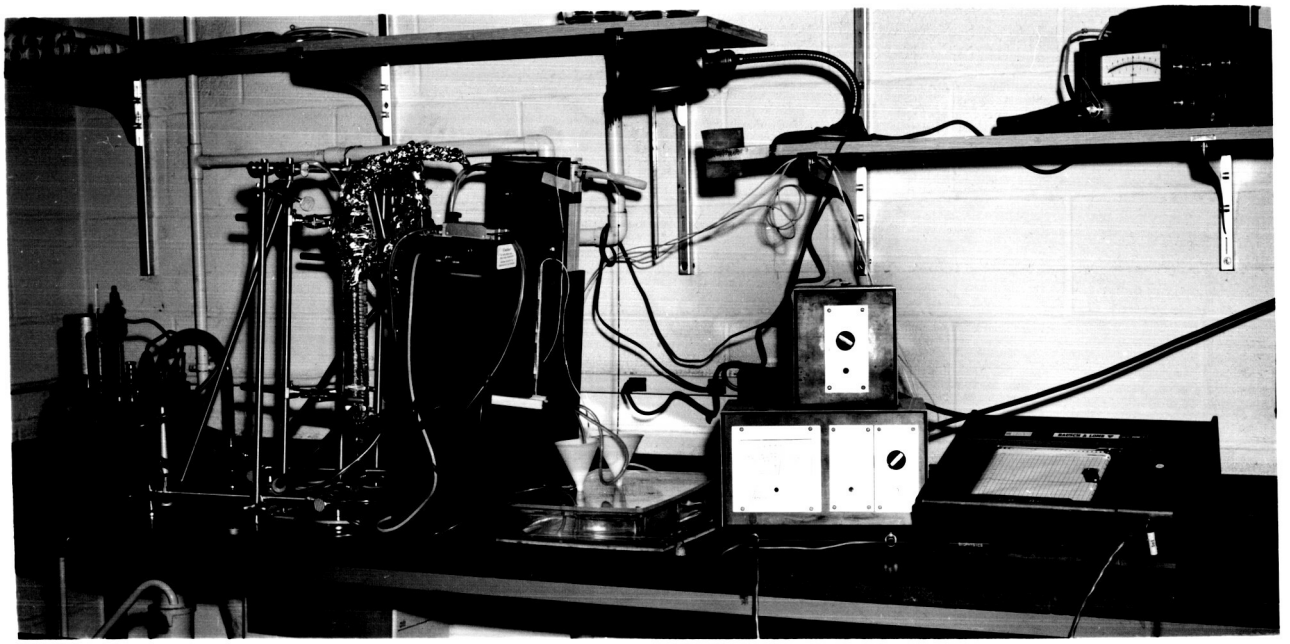

Figure 2

Figure 3

TABLE 1

	Samples				Controls						
(1) Date of Fert.	(2) % Developed	(3) Temperature Avg.	(4) Max.	(5) Min.	(6) % Controls Developed	(7) Temperature Avg.	(8) Max.	(9) Min.	(10) Ratio 2 to 5	(11) Difference 6 to 2	(12) Comments	(13) Finger Bowl
1/21/67	8%	23.0	24.0	22.9	31%	21.0	22.2	20.9	.26	+23%	temp. of sample & control differ	77%
1/27/67	24%	22.1	22.9	21.3	46%	20.9	20.3	21.5	.52	+22%	"	82%
2/03/67*	0%	20.8	21.4	20.1	0%	20.0	20.7	19.4	0.00	0%	"	90%
3/08/67	14%	+25.0			57%	23.8	24.4	23.1	.25	+43%	"	96%
3/17/67	77%	21.4			62%	20.5			1.24	-15%	"	69%
3/21/67	31%	20.8	21.6	20.0	85%	22.5	23.3	21.6	.36	+54%	"	80%
3/31/67	46%	21.4			46%	21.1			1.00	0%	constant temp. room	67%
4/03/67	23%	21.6	21.9	21.0	62%	21.3	21.8	20.8	.37	+39%	faster pump, touching	65%
4/07/67	8%	20.9	21.9	20.2	54%	21.1	21.9	20.4	.15	+46%	touching	95%
4/10/67	0%**	21.2	22.2	20.5	38%	21.0	21.4	20.6	0.00	+38%	"	50%
4/14/67	77%	20.9	22.0	20.7	62%	20.5	20.5	20.4	1.16	-15%	"	57%
4/16/67											incomplete run, overflow	
4/24/67	69%	20.7	21.4	20.3	69%	20.8	21.4	20.2	1.00	0%	increased gap, painted pole piece	78%
4/27/67	15%	20.9	21.4	20.4	50%	20.9	21.2	20.5	.30	+35%		48%
5/01/67	31%	20.9	20.9	20.6	15%	20.8	21.0	20.7	2.67	-16%		77%
5/04/67											pump broke. all embryos dried up. appeared to be hatched at 100% rate	100%
5/09/67	23%	20.9	21.4	20.3	100%	20.8	21.3	20.3	.23	+77%		90%
5/12/67	92%	20.9	21.6	20.4	92%	20.8	21.4	20.5	1.00	0%		90%
5/16/67	0%	20.8	21.5	20.3	23%	20.9	21.7	20.5	0.00	+23%		-
5/19/67	0%	20.8	21.0	20.4	54%	20.8	21.1	20.2	0.00	+54%		-
5/23/67	31%	20.8	21.3	20.5	92%	20.8	21.1	20.6	.34	+61%		95%
Average	30.2%				55.4%					+25.2%		78%

* stainless steel containers

** 46% at neural groove

compartments was, from time to time, welling up and touching the pole of the magnet. Before the April 24th run this problem was solved by increasing the gap between the egg holder and the magnet. Because of the need to increase the size of the gap by about 1/8" the field in the original gap was first rechecked with a Bell gaussmeter on April 19.

The field at the mid-level, M, where the embryos are, was 10,000 oe; at the surface A it was 13,500 oe and near the upper surface U it was 6000 oersted (see Figure A1). About .120" was then removed from the upper pole piece as shown in Figure A2 and it was reinserted. The magnetic measurements on this configuration made April 21, showed that at the lower surface A' the field was now 12,600 oe and 1/4" above it, 7,400 oersted. At the midpoint 1/8" above the lower pole piece surface, the field is therefore now approximately 10,000 oersted and has a gradient of 8,350 oe^2/cm, making

$$H \frac{dH}{dx} = 8.35 \times 10^6 \ oe^2/cm$$

not substantially different from the original value as measured by the General Electric Company which had a slightly higher H but lower dH/dx. (See Figure A2.)

No obvious trouble was encountered from this time on. It should be pointed out that the experimental results may or may not have been affected by these conditions; the occurrence of various incidences are shown in the comments column (Table 1).

Some of the hatched embryos from both sample and control groups were allowed to continue growing and their continued development as well as their swimming (motion) was observed. Movie records were made to analyze their ability to swim in detail. Probably because of the restricted size of the holders, some tadpoles had slightly deformed tails, but no obvious differences between the two groups was found, i.e. between the magnet and control group.

III. RESULTS

The results showed a surprising cessation of embryological development at an early stage in a high percentage of the exposed samples.

The percentage of developing embryos are shown completely in Table 1, along with the records of the temperatures in both containers. An average of 55.4% of the controls developed through the hatching stage while only 30.2% developed in the gradient magnetic field and 78% developed in the finger bowls. There were two finger bowls. One simply was filled with nutrient solution, the other had the nutrient solution from the magnet sample drop into it continuously. No differences were observed for these two cases and the results of the second are reported in column 13 (Table 1). This may indicate that there were factors in the container size or material* that inhibited development. In order to see if the difference

* i.e. material actually in contact with the embryos.

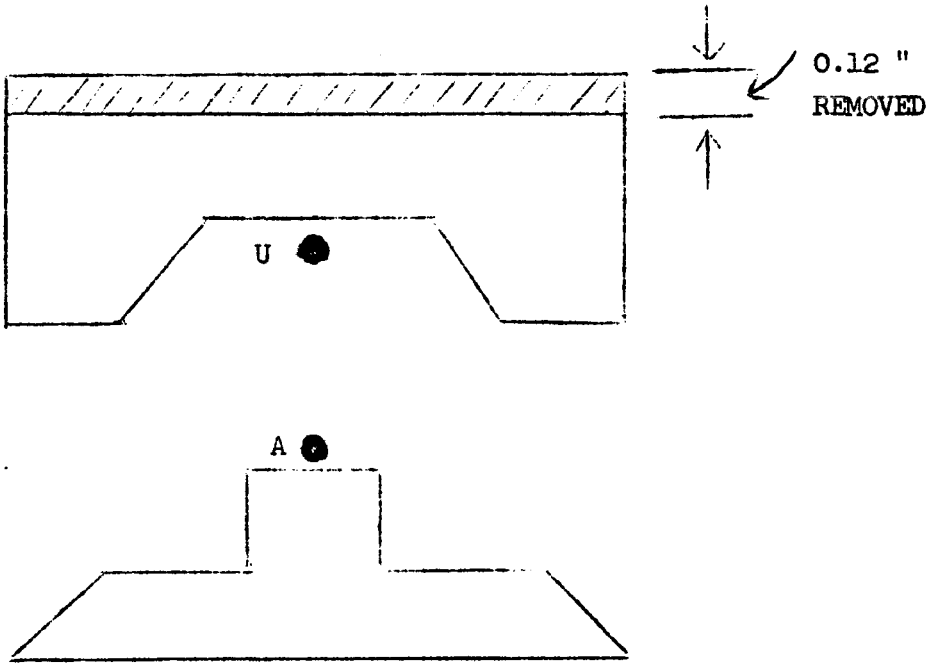

Figure A1

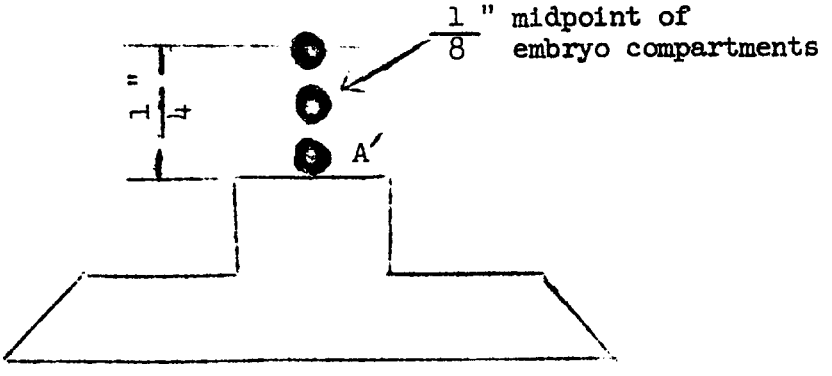

Figure A2

between the percentages of developing control and sample embryos was signficant, standard deviation and chi square tests were performed on the experimental results. The results of both tests showed the experimental results to be only moderately significant. The percentage difference is approximately equal to one standard deviation, and the probability of these results arising by pure chance is about 20%.

It was found that all the non-developing embryos in both the sample and the control containers stopped their development during the formation of the blastula or at gastrulation (see Table 2). In those that appeared to have stopped during the blastula stage, the exact stage was difficult to determine since they appeared white and decayed. Others that continued through the formation of a blastula, but not to the hatching stage, had large masses of yolk material that did not invaginate during gastrulation. It is interesting to note that all non-developing embryos from a particular run, whether sample or control, stopped development at the same stage. At present, histological studies are being done to determine more definitely the stage and the cause of cessation of development.

IV. PRESENT AND FUTURE WORK

At the present time, as was mentioned in the previous section, histological studies are being carried out on fixed non-developing embryos to determine the differences between them and the developing embryos. The slides, when completed, will be compared to the slides of embryos from all stages made last year. (see Semi-Annual Report, May 12, 1966). The method of preparation for embedding, cutting and staining is essentially the same as stated in that report except that Bouin's Fluid alone is being used for fixation (as opposed to a 50-50 mixture of Bouin's and dioxane last year) and that tetrahydrafuran (rather than dioxane) is being used for dehydration. This method was found to make the embedded embryos less brittle.

The biochemical method of analyzing iron content which was developed last year (see Appendix to letter to Dr. Huertas, September 23, 1966) will also be used on these fixed non-developing eggs to determine whether or not there is any deviation from the normal ones tested by this method last year. Eggs to be used for this purpose have been fixed in 5% formalin as described in the final report dated January 30, 1967.

Since the results from the 19 runs thus far have not proved to be statistically significant, more runs are being planned as soon as eggs are available.

Table 2

Date of Fertilization	Approximate Stage of Non-developing samples	Approximate Stage of Non-developing Controls
1/21/67	blastula	blastula
1/27/67	blastula	blastula
2/03/67*	blastula or gastrula	blastula or gastrula
3/08/67	blastula	blastula
3/17/67	blastula	blastula
3/21/67	blastula	blastula
3/31/67	blastula	blastula
4/03/67	blastula	blastula
4/07/67	blastula	blastula
4/10/67	blastula	blastula
4/14/67	blastula	blastula
4/16/67	blastula	blastula
4/24/67	gastrula	gastrula
4/27/67	gastrula	gastrula
5/01/67	blastula	blastula
5/04/67	-	-
5/09/67	gastrula	gastrula
5/12/67	gastrula	gastrula
5/16/67	gastrula	gastrula
5/19/67	gastrula	gastrula
5/22/67	gastrula	gastrula

* stainless steel containers

Figure and Table Captions

Figure 1 Two-hour embryos being placed in compartments of holder in magnetic field

Figure 2 Complete set-up, showing magnet, holders in place, cooling apparatus for circulating medium, and temperature measuring and recording devices

Figure 3 Looking through space between top of holder and bottom of magnet pole. Space had to be increased because of nutrient welling up in container and touching magnet

Table 1 Data for all 19 experiments shows:

a. percentages of embryos surviving to hatching stage in sample and control containers and in finger bowl

b. temperature records for sample and control containers

c. problems encountered in each experiment (comments)

Table 2 Shows stages which non-developing embryos reached before stopping development. Note that both control and sample non-developers stopped at the same stage in each experiment even though the numbers not developing were different